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INDUCTION AND TERMINATION OF PUPAL DIAPAUSE IN *SARCOPHAGA* (DIPTERA: SARCOPHAGIDAE)[1]

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Numerous studies on the bionomics of flies report the existence of a third-instar- or pupal diapause, but knowledge of the environmental factors regulating diapause is sparse, and information on the physiology of fly diapause is practically non-existent. In flesh flies of the genus *Sarcophaga*, experiments on diapause have been restricted to studies by Roubaud, 1922; Fraenkel and Hsiao, 1968a, 1968b; Denlinger, 1970, 1971b, 1972; Saunders, 1971; Vinogradova and Zinovjeva, 1971.

Diapause in *Sarcophaga* is most commonly observed in the young phanerocephalic pupa, a stage in which adult development has not been initiated. Occasional *S. bullata* pupae diapause in a slightly more advanced state, in which adult antennal discs are visible (Fraenkel and Hsiao, 1968a), and preliminary observations (William Downes, Michigan State University, personal communication) indicate that a larval diapause may also exist in several *Sarcophaga* species found in southern United States.

Inadvertent exposure of *S. argyrostoma* larvae to cold temperature and short daily photophase first revealed the importance of these factors in pupal diapause induction (Fraenkel and Hsiao, 1968a); however, larvae of *S. bullata* were not responsive to photoperiod. More recent experiments on pupal diapause in *S. crassipalpis* have demonstrated the extreme importance of the photoperiod received by embryos developing within the female's uterus (Denlinger, 1970, 1971b), and Saunders (1971) has reconfirmed a larval sensitivity to temperature and photoperiod in *S. argyrostoma*.

Although many of Roubaud's (1922) experiments were based on only one pupa, diapause did not appear to be terminated by high temperature or by mechanical or chemical means; however, chilling appeared to cause an early termination of diapause. An acceleration of diapause termination in *Sarcophaga* has also been accomplished with ecdysone injections (Fraenkel and Hsiao, 1968b).

The present investigation was carried out to define further the environmental parameters responsible for induction and termination of pupal diapause in flesh flies. Comparisons are made between different species and strains of *Sarcophaga*.

Materials and Methods

Flies used in the experiments represent three species: *Sarcophaga argyrostoma* (Robineau-Desvoidy), *S. bullata* Parker, and *S. crassipalpis* Macquart. Labora-

[1] This paper is based on material contained in a thesis for the doctoral degree at the University of Illinois at Urbana-Champaign.

tory cultures of *S. argyrostoma* and *S. bullata* were from the same strains used by Fraenkel and Hsiao (1968a). Wild strains of *S. bullata* started from single females collected in Champaign County, Illinois, and St. Louis County, Missouri, were also used. Data on the wild strains of *S. bullata* were collected during the first year of laboratory culture. The stock of *S. crassipalpis* came from progeny of three wild-caught females collected in Champaign County, Illinois, five years before this investigation. Stocks were maintained in an insectary at 26 ± 1° C in continuous light.

Environmental cabinets (Percival Refrigeration Co.) were used to maintain a temperature of 25 ± 0.5° C. Temperatures of 17 ± 1.0, 26 ± 1.0 and 28 ± 1.0° C were maintained in walk-in temperature cabinets. The vicinity of the experimental animals received a light intensity range of 10–45 lux during the photophase.

Groups of about 100 adult flies were kept in 1 ft square aluminum framed, screened cages (Cornell Equipment Co.). Sucrose and water were provided throughout adult life; pork liver was provided as a source of protein from the first day following adult eclosion until the seventh day.

All three species are ovoviviparous. Embryonic development occurs within a sac-like uterus, and, at 25° C, eggs hatch in the uterus by the eleventh day of female adult life. In the absence of liver, larvae are not deposited by the females. Collections were made by snipping off the tip of the female's abdomen on the eleventh day and extruding the larvae. Thus, a uniform starting time was achieved for progeny of different females.

The number of progeny produced in the first ovarian cycle differs among the species and strains examined. The mean numbers of progeny/female are as follows: *S. argyrostoma*, 30.2; *S. crassipalpis*, 61.7; *S. bullata* (lab), 55.6; *S. bullata* (Mo.), 96.4; *S. bullata* (Ill.), 103.4.

Larvae were reared on fresh pork liver homogenized in a Waring blender. Progeny of single females were reared in separate packets made of .001 gauge aluminum foil. Boats with a area of 8 cm × 4.5 cm were produced by forming a piece of foil 20 cm × 23 cm around a block of wood. Liver was weighed into each boat in order to provide about 0.5 g/larva. After addition of the larvae, the top of the packet was compressed and the excess aluminum foil was cut off, leaving a narrow slit open at the top. The packets containing the liver and larvae were placed on 1 cm sifted sawdust within a 16 oz squat container (Dixie Products, No. 2186-SE). Mass rearings of 400–600 larvae for the stock cultures were carried out in 11 cm × 9 cm foil boats. When the third instar larvae cease feeding they crawl out of the aluminum foil packets and puparíate in the sawdust. The rearing method serves to reduce the odor associated with the meat and has the advantage of allowing easy separation of pupae from the rearing medium.

The incidence of diapause is more consistent among different batches when small numbers of larvae such as the progeny of single females are reared together. In mass rearing the temperature in the microclimate produced by the liver and larvae rises many degrees above ambient temperature and thus may cause variation among batches. To minimize variation, all results are based on data collected from separate packets containing progeny of single females.

The incidence of diapause was determined by removing the anterior cap of the puparia to examine the pupae for the presence of imaginal development. In

experiments reporting the time required for diapause termination, pupae were examined every 10 days. From knowledge of the time required for attainment of various post-diapause developmental stages, the date of the first visible signs of diapause termination (antennal spots observed by Fraenkel and Hsiao [1968a]) could easily be estimated. Although respiration studies show that breaking of diapause can be detected several days before the appearance of the first morphological signs of adult development (Denlinger, Willis, and Fraenkel, 1972), the day adult antennal discs became visible has been designated as the day of diapause termination.

Results

Role of adult and larval photoperiod and temperature on diapause induction

The incidence of diapause observed under different adult and larval photoperiods and temperature is recorded in Table I. There are no significant differences in the effects of 8, 10 or 12 hour photophases; therefore, results from these photophases are combined, and are denoted in Table I as "short."

Diapause is averted in all species and strains if a long photophase or continuous light is provided for both the adult mothers and larval offspring at 25° C.

Table I

Influence of maternal adult and larval photoperiod and temperature on incidence of pupal diapause in Sarcophaga *species and strains*

Experimental conditions				Species and strains									
Maternal adults		Larvae		*S. argyrostoma*		*S. crassipalpis*		*S. bullata* (lab)		*S. bullata* (Mo.)		*S. bullata* (Ill.)	
Photo-period	Temp.	Photo-period	Temp.	No. pupae	% dia-pause	No. pupae	% dia-pause	No. pupae	% dia-pause	No. pupae	% dia-pause	No. pupae	% dia-pause
24:0	25° C	24:0	25° C	875	0	1089	0	743	0	449	0.2	1172	0
"	"	short	"	118	0	38	0	689	0	368	0	294	0
"	"	"	17	342	84.3	1138	4.0	220	0.4	311	11.6	478	32.6
17:7	"	17:7	25	—	—	872	0	—	—	1666	0	1618	0
"	"	short	"	—	—	170	1.2	—	—	492	0	426	0
short	"	24:0	"	240	0	1242	9.1	280	0	493	0	212	0
"	"	17:7	"	53	0	237	9.3	643	1.9	143	11.9	380	11.0
"	"	short	28	86	15.1	372	57.0	112	6.2	465	8.2	404	14.8
"	"	"	25	183	31.8	959	85.7	621	22.5	1502	87.3	2537	73.4
"	"	"	17	234	100	568	100	542	73.8	150	100	179	100

"Maternal" photoperiod is important for all of the flies; under the same larval conditions the diapause level is always higher following maternal exposure to short day. Thus, no diapause occurs when larvae are reared at short day and 25° C without short day exposure of the maternal adults, and larvae reared at 17° C have a higher diapause incidence when their mothers are exposed to short day. Maintaining short-day adults of *S. crassipalpis* and *S. bullata* (lab) at a higher temperature (28° C) does not appear to alter the incidence of diapause in short-day progeny reared at 17, 25 and 28° C (each $N \geq 287$ pupae).

The important role played by the adult photoperiod is supplemented with a reinforcing role of photoperiod acting on larvae reared at 25° C. If only the maternal adults receive a short daily photophase, the level of pupal diapause is low. The diapause level increases greatly with larval exposure to short day. Progeny of short day *S. bullata* (Ill.) adults reared in continuous darkness at 25° C (N = 183 pupae) show a great decrease in diapause incidence as compared to larvae reared at short day (3% in contrast to 73%).

The effect of photoperiod is influenced by the temperature at which the larvae are reared. Under experimental conditions in which only the larval rearing temperature is variable, the incidence of diapause increases with a decrease in temperature. Thus, the maximum diapause response for all flies occurs with a combination of short days for the adults and short days and low temperature (17° C) for the larvae.

There are differences between the species and strains of *Sarcophaga*. In the absence of adult short photoperiod, *S. argyrostoma* is more sensitive than the other species and strains to the larval diapause-inducing factors of short photoperiod and low temperature. The laboratory strain of *S. bullata* enters diapause at the lowest rate under each of the experimental conditions. Differences also exist between the two wild populations of *S. bullata*. When the entire life cycle is spent under a short daily photophase at 25° C, the level of diapause is greater for the strain collected in Missouri (see also Fig. 1).

Critical photoperiod

Samples of *S. bullata* from Missouri and Illinois were maintained throughout the life cycle at 25° C under different photoperiod regimens. Each point in Figure 1 represents the mean diapause incidence in the progeny of 12–14 females; 925–1666 pupae were produced by these females.

The transition between diapause induction and continuous development occurs abruptly at a daily photophase of 13½ hours for both populations. With a daily photophase shorter than the critical photoperiod, the diapause level is high. The unnatural conditions of less than an 8-hour photoperiod caused a decline in the diapause response of *S. bullata* (Ill.).

Effect of water content of larval medium on diapause induction

S. crassipalpis larvae taken from the same lot of adults maintained at 25° C-12 hour photophase were reared either on the standard medium of homogenized fresh pork liver or on a mixture of the medium + 10% water (40 g liver + 4 ml H_2O/progeny of 1 female). Larvae were reared at 25° C-12 hour photophase in the same incubator. Progeny reared on the standard medium produced an 86.1% (N = 553) incidence of pupal diapause; addition of 10% water to the medium produced a 95.7% (N = 716) incidence of diapause (Pearson $\chi^2 = 29.69$, d.f. = 1, $P < 0.01$).

Effect of adult protein deprivation on diapause

Adults of *S. argyrostoma*, a species capable of producing its first batch of progeny without protein, were maintained on a protein-free diet (Denlinger,

1971a); other flies from the same lot were provided protein. The incidence of diapause in the progeny of the two groups was compared (81.6% without protein, N = 136; 84.3% with protein, N = 186 pupae). The test failed to show a significant difference in progeny diapause level (Pearson $\chi^2 = 0.29$, d.f. = 1, $P > 0.50$).

Influence of sex on diapause induction

Comparison of the sex ratios in groups of *S. crassipalpis* representing various levels of diapause incidence shows a tendency for males to enter diapause at a higher rate than females. The sex ratio approaches 1 : 1 for batches representing 0% diapause (51% males, N = 993) and 100% diapause (48% males, N = 761).

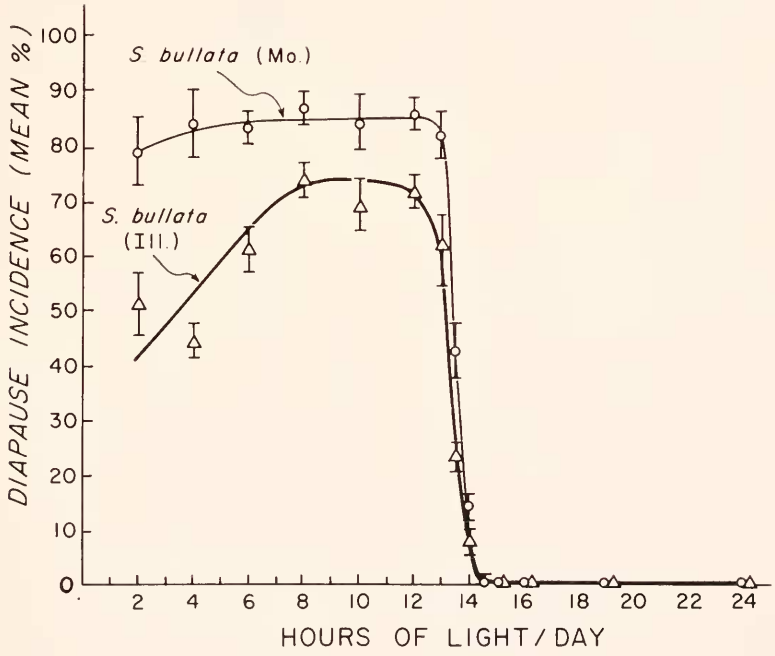

FIGURE 1. Critical photoperiod at 25° C in samples of wild populations of *Sarcophaga bullata* from Illinois and Missouri. Each point represents the mean (± S. E.) diapause incidence in progeny of 12–14 females (925–1666 pupae).

With partial diapause incidence the sex ratios of the diapause and non-diapause fractions deviate significantly from 1 : 1. In batches representing < 10% diapause, 87% of diapausing flies were males (N = 102). The tendency for males to enter diapause more readily than females is further exemplified by the sex ratio of flies developing without a diapause in batches representing > 90% diapause (25% males, N = 124). Observations on the other species of *Sarcophaga* have revealed a similar pattern.

Delay of pupariation in diapause-committed larvae

Flies reared under diapause-inducing conditions pupariated later and over a greater span of time than flies reared under non-diapause conditions. Adult flies originating from one mass-reared batch were maintained under either a short or long daily photophase at 25° C. and all larvae were maintained at a short photophase at 20° C. At 24 hour intervals following collection of the larvae, the rear-

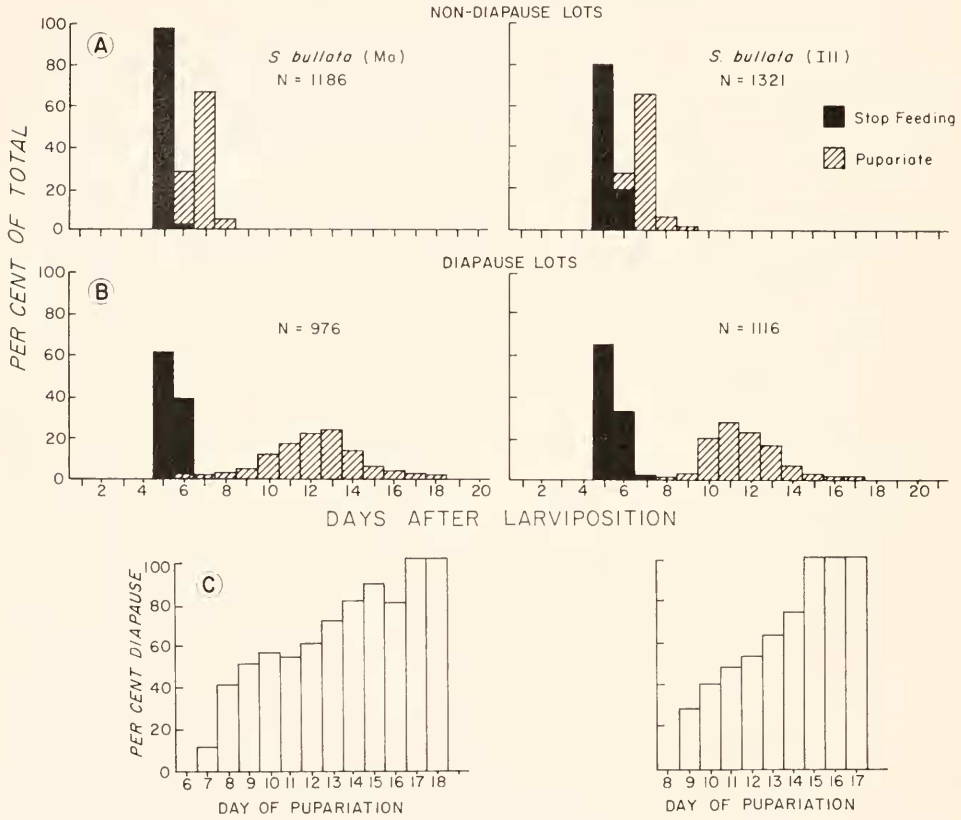

FIGURE 2. Delay of pupariation and correlation with incidence of pupal diapause in *Sarcophaga bullata* reared under diapause-inducing conditions. All bars are continuous to the base line.

ing containers were examined, and the number of larvae having left the liver and the number having pupariated within the time interval were recorded. Pupae from diapausing lots were combined on the basis of the day of pupariation and were later examined to determine the incidence of diapause.

Data for *S. bullata* (Mo.) and *S. bullata* (Ill.) are recorded in Figure 2. Larvae from both the non-diapausing (long-day) lots and diapausing (short-day) lots cease feeding and leave the liver at approximately the same time. In the non-diapausing lots (Fig. 2A), pupariation follows immediately; by the seventh day following larviposition, over 90% of the larvae have pupariated. In con-

trast, pupariation in diapausing lots (Fig. 2B) is delayed and occurs over a period of many days. Examination of pupae collected on each day revealed an increase in the incidence of diapause with an increase in the time of pupariation (Fig. 2C). Studies with *S. crassipalpis* and *S. argyrostoma* have shown a similar relationship of pupariation and diapause incidence; however, in these species the delay is not quite so long as that observed with the wild strains of *S. bullata.*

Effect of temperature on diapause termination

Pupae held under a daily 12-hour photophase were maintained throughout diapause at either 17 or 25° C; the time required for diapause termination at the two temperatures is recorded in Table II. A multiple regression analysis indicated

Table II

Duration of pupal diapause at 17 and 25° C for species and strains of Sarcophaga. *Means followed by the same letter are not significantly different at 5% level.*

Species	Diapause duration (days) at constant temperature				Minimum diapause duration (days) at combination of temperatures*	
	17° C		25° C		Estimated no. days at each temperature	Mean total days
	No. pupae	Mean ± S.E.	No. pupae	Mean ± S.E.	17°/25°	
S. argyrostoma	92	227.4 ± 1.3	52	91.7 ± 1.1	35/35	70
S. crassipalpis	146	117.9 ± 0.4[a]	216	69.8 ± 0.7[b]	20/20	40
S. bullata (lab)	105	61.3 ± 1.1	98	70.6 ± 1.4[b]	20/20	40
S. bullata (Mo.)	84	117.4 ± 1.3[a]	108	106.5 ± 1.6	45/35	80
S. bullata (Ill.)	153	174.1 ± 2.5	63	133.3 ± 2.7	55/40	95

* See text.

a highly significant interaction of species and strains with temperature ($F = 351.4$, d.f. = 4 and 1109, $P < 0.0001$). Temperature, species and strains, and their interaction account for 88.2% of the variation in duration of diapause. Comparison of all possible pairs of means by the SNK test, using MS error (= 329.6), indicates that all differences are significant except those designated in Table II. The two species with insignificant differences in diapause duration at one temperature are sgnificantly different at the alternate temperature.

With the exception of the laboratory strain of *S. bullata,* all the flies show a decrease in the duration of diapause with an increase in temperature. This relationship is further exemplified in *S. crassipalpis,* which has a mean diapause duration of 56.6 ± 1.4 days (N = 87) at 28° C. In this species duration of diapause was regressed on temperature (at 17°, 25°, 28° C), with temperature coded as °C minus 25°. The analysis of variance of the regression demonstrates a highly significant, negative curvilinear relationship between diapause duration and temperature (linear component with $F = 1691.5$, d.f. = 1, M.S. = 277138, $P < 0.0001$; quadratic component with $F = 8.1$, d.f. = 1, M.S. = 1322, $P < 0.001$; Residual

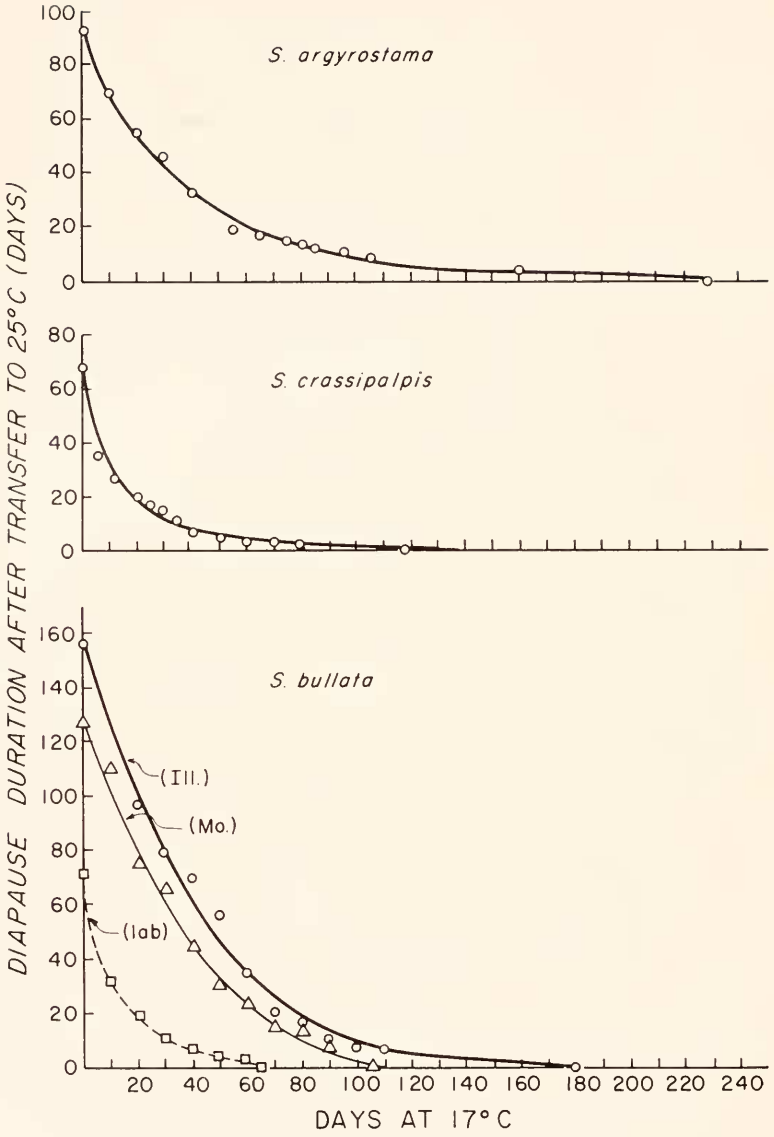

FIGURE 3. Duration of diapause in *Sarcophaga* species and strains at 25° C after various lengths of time at 17° C. Points on axes are from Table II; all other points represent the mean of 25–30 pupae.

d.f. = 446, M.S. = 164). The estimated regression equation is $\hat{Y} = 69.41 - 4.78X + 0.16X^2$, where X is coded temperature.

By providing diapausing pupae with a combination of 17 and 25° C, the duration of diapause decreases to a lower level than is observed with constant exposure to either temperature. Groups of 25–30 pupae were transferred from 17° to 25°

at 10 day intervals following pupariation; the mean number of days required to break diapause after transfer to 25° C is recorded in Figure 3. Points on the axes of the graphs were obtained from data on diapause duration at constant temperatures (Table II). The shape of the curve is similar for all of the species and strains although there are differences in the points of interception with the axes. The minimum number of days to break diapause has been estimated by calculating the point of the curve which is closest to the origin. The combinations of days which provide the minima are recorded in Table II. The duration of diapause can be reduced by 20–40 days by initial exposure to 17° C and subsequent transfer to 25° C.

Effect of non-temperature factors on diapause termination

To test the possibility that photophase influences the duration of diapause, lots of diapausing *S. crassipalpis* pupae at 25° C were divided into halves which were kept under a daily 12 hour photophase or transferred to a daily 17 hour photophase 10 days after pupariation. Of the 21 replications of the experiment performed, 3 replications (containing a total of 1044 pupae) were selected randomly for a multiple regression analysis incorporating terms for photophase, lots, and their interaction. Interaction was not significant and was dropped from the model. In the reduced model the effects of both lots and photophase were significant at levels of 0.1% and 5%, respectively. An increase of photophase from 12 to 17 hours increased the diapause duration 1.8 days. Lots and photophase together accounted for only 4.7% of the variation in duration of diapause and photophase alone for less than 1%. Even if the very slight difference in duration of diapause under the short and long photophases is not accounted for by an unassumed bias, the difference produces little biological effect.

The duration of diapause in diapausing *S. crassipalpis* pupae from lots with a low incidence of diapause (36.4%, $N = 212$ pupae) was compared with lots having a high incidence of diapause (94.2%, $N = 248$ pupae). The 2.3 day difference in latency (high > low) found to be significant with the Student's t test is not greater than can be attributed to the variation among lots.

Discussion

Stimuli for induction

Adverse winter conditions in the temperate regions have channeled the evolution of a pupal diapause in flesh flies of the genus *Sarcophaga*. As has been demonstrated in many invertebrates (reviews by Lees, 1955; de Wilde, 1962; Danilevskii, 1965; Beck, 1968), environmental cues of short photoperiod and low temperature are primarilly responsible for programming diapause in flesh flies.

Fraenkel and Hsiao's (1968a) and Saunders' (1971) experiments with larvae of *S. argyrostoma* have demonstrated the role played by short photoperiod and low temperature on diapause determination; larval photoperiod was not shown to influence diapause in *S. bullata*. My experiments confirm their results, but supplement their data with information on the extreme importance of maternal adult photoperiod among all *Sarcophaga* examined. If only the larvae receive

a short daily photophase at 25° C, the pupae will not enter diapause. If adults as well as larvae receive a short-day stimulus, a moderate incidence of pupal diapause occurs, even in *S. bullata.* Maximum diapause response occurs when adult and larval short-day stimuli are supplemented with larval exposure to 17° C. Investigation of the role played by the adult photoperiod has shown that the photoperiodic stimulus acts directly on the embryos developing within the uterus of the ovoviviparous females (Denlinger, 1970, 1971b).

At 25° C, photoperiods longer than 13½ hours permit continuous development in populations of *S. bullata* from Missouri (38°30′ N, 90°30′ W) and Illinois (40°15′ N, 88°15′ W). Shorter photoperiods which occur naturally in Missouri and Illinois are diapause-inducing. In *S. bullata* (Ill.) unnaturally short photoperiods produce a gradual decline in diapause response. A laboratory strain of *S. argyrostoma* examined by Saunders (1971) has a critical photoperiod of 13½–14 hours at 15° and 20° C. Differences in the critical photoperiods of populations from different geographic regions are commonly observed (Danilevskii, 1965); however, no such difference has been observed across the small latitudinal distance of 1°45′ for *S. bullata.*

Food quality and quantity may exert an influence on diapause in some insects. The physiological or ecological meaning behind the 10% increase in diapause with an increase in the moisture content of the larval medium in *S. crassipalpis* remains unknown. Literature on the role of water for diapause termination is abundant (Beck, 1968), but the role of water in diapause induction is less well documented. An opposite effect of moisture content was observed in *Lucilia sericata;* dry meat produced a greater incidence of third-instar diapause (Cousin, 1932; Mellanby, 1938). Diapause in the progeny of *S. argyrostoma,* a species capable of producing viable eggs in the absence of an adult protein meal (Denlinger, 1971a), was not affected by adult protein deprivation. It is not surprising that maternal nutrition does not influence diapause since there is no evidence that the female passes nutritive material to the embryos within her uterus, as was suggested by Cholodkovsky (1908); the fact that embryos can be cultured *in vitro* (Denlinger, 1971b) emphasizes the physiological independence of the embryos.

Sexual differences in tendency to enter diapause are especially apparent from examination of lots exhibiting a partial diapause response. Males of *Sarcophaga* enter diapause at a higher rate than females. *Lucilia caesar,* a species with a larval diapause, shows a similar tendency (Ring, 1971). The sexual differences in *Sarcophaga* imply a slightly different threshold for photoperiod and temperature response. Receiving a short-day stimulus for only the embryonic period (produces 10% diapause) is apparently insufficient for diapause induction in most females. A temperature threshold appears to be involved also since at short day-25° C (produces 85% diapause) three times as many of the non-diapausing flies are females; lowering the temperature to 17° C produces 100% diapause and an even sex ratio in the diapausing pupae.

Characteristics of diapause-committed larvae

Induction of pupal diapause in *Sarcophaga* is strongly correlated with another developmental feature, delay of pupariation. There is a general tendency for prediapause development to be slower than nondiapause development (Beck,

1968). This retardation appears to be exemplified by *Sarcophaga* because pupariation in diapause-committed lots of larvae occurs several days after pupariation in non-diapause lots. Within a given batch of short-day larvae the incidence of diapause increases directly with the time of pupariation. The retardation of development is not evenly distributed throughout larval life, but occurs after feeding has ceased. During the post-feeding period larvae wander in search of a dark, dry area for pupariation; the crop is gradually cleared during this time.

The reason behind such a delay is unknown. An adaptive advantage may be afforded a diapause-destined larva if it has more time to find a suitable hibernaculum. On a physiological level this phenomenon may be pointing to an early hormonal difference between larvae which will enter pupal diapause and those which will complete development immediately. The period of delay in the post-fed third instar occurs at the same developmental stage as the larval diapause in numerous other Diptera. Perhaps the longer larval life in diapause-committed *Sarcophaga* offers an evolutionary clue to the similarity of larval and pupal diapause.

Experimentally, pupariation can be delayed in *Sarcophaga* by exposure of the full-grown larvae to moisture (Evans, 1935; Ohtaki, Milkman and Williams, 1968; Zdarek and Fraenkel, 1970) and by larval injections of juvenile hormone (Srivastava and Gilbert, 1969). However, delaying pupariation by keeping full-grown larvae wet for several days is not sufficient to induce pupal diapause (Denlinger and Zdarek, unpublished observation). Arrest during pharate adult development can be brought about with larval injections of juvenile hormone in *S. bullata* (Srivastava and Gilbert, 1969). Perhaps these results may be interpreted as a diapause-like event. The fact that the arrest occurs at different life stages than is observed with naturally occurring diapause may not be too critical, since occasional *S. bullata* pupae also diapause in a slightly later developmental stage.

Lowering the larval rearing temperature delays pupariation, and concurrently there is an increase in the incidence of diapause (Saunders, 1971). Saunders attributes the increase in diapause not to a direct temperature effect but to an increase in the number of days of exposure to the short-day regimen. This hypothesis does not explain a difference in the time of pupariation between larvae from long-day adults and larvae from short-day adults which are reared side by side at 26° C-12 hour photophase as was done in this investigation. A comparison of these two groups of larvae reared at the same temperature indicates that the larva's commitment to pupal diapause is the cause of the delay and not the result of it. That larvae which will enter diapause receive additional light cycles due to a delay in time of pupariation is inconsequential to the induction of diapause in a species such as *S. crassipalpis* where the late larval life is unimportant for diapause determination (Denlinger, 1971b).

Stimuli for termination

The effect of "chilling" reported in *Sarcophaga* by Roubaud (1922) and Fraenkel and Hsiao (1968a) is confirmed by the persent observation on the acceleration of diapause termination by transferring pupae from 17 to 25° C. Physiologically the effect of change in temperature may result from interaction of various biochemical processes having different temperature optima.

The observed relationship between temperature and diapause termination provides interesting insights on the nature of diapause in *Sarcophaga*. Unlike many species which cannot terminate diapause at a constant temperature, *Sarcophaga* pupae entering diapause at constant temperatures ranging from 13.5° C (Fraenkel and Hsiao, 1968a) to 28° C can utlimately terminate diapause at that same temperature. The rate of completion for the physiological processes of diapause accelerates with temperature. Although such a relationship is common for most biological processes, Williams (1956) found no apparent rate change for pupal diapause termination in *Hyalophora cecropia* held at constant temperatures ranging between 10–25° C.

When diapausing Cecropia silkworms are "chilled" at constant temperatures ranging from 2½ to 20° C and then transferred to 25° C, the time required for initiation of adult development is reported to remain constant with the first few weeks of chilling and then to drop off precipitously (Williams, 1956). The initial plateau of the curve, a period which Williams attributes to a threshold reaction for reactivation of the brain, is not observed when *Sarcophaga* pupae are transferred to 25° C after various periods of "chilling" at 17° C. In all five of the species and strains of *Sarcophaga* examined, the duration of diapause begins to fall off immediately with time. Differences in the diapause intensity of the two insect groups or the fact that the Cecropia pupae received ten weeks of 25° C prior to the chilling experiments may account for the observed differences. The ability of ecdysone to terminate pupal diapause in both *H. cecropia* (Williams, 1946) and *Sarcophaga* (Fraenkel and Hsiao, 1968b) implies a similar hormonal basis for the diapause state.

Dependency on temperature rather than photoperiod as a cue for the termination of diapause is ecologically appropriate for *Sarcophaga* since the pupae are normally buried under the surface of the soil. In this situation photoperiod would not provide a readily accessible seasonal cue, whereas soil temperature could provide an accurate assessment of the advent of the favorable season in temperate regions.

It was thought that the duration of diapause may vary between lots with a low and high incidence of diapause. Such a difference in the intensity of diapause is not apparent. On an individual basis diapause is not a graded response but an "all or none" phenomenon.

Species and strain differences

Experiments with *S. argyrostoma, S. crassipalpis,* and three strains of *S. bullata* demonstrate the variability of the diapause characteristics among species and strains. Unlike the other flies, *S. argyrostoma* responds with a high incidence of pupal diapause if the larvae are reared at 17° C-12 hour photophase without an adult short-day stimulus; the role of adult photoperiod is more important in other species. In most cases the direction of response to temperature and photoperiod observed is the same but differs in degree. Differences in the duration of diapause exist among the *Sarcophaga;* a difference exists between the wild *S. bullata* strains from Illinois and Missouri, and a very pronounced difference exists between the wild strains and the laboratory strain of the same species. Many years of selection and inbreeding in the laboratory under continuous light and constant temperature

may account for the aberrance of this strain. The results with the laboratory strain of *S. bullata* suggest the need for caution in projecting to the natural world results obtained from highly inbred laboratory animals.

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Summary

1. In temperate regions species of *Sarcophaga* overwinter in pupal diapause. The environmental control of diapause is investigated in *Sarcophaga argyrostoma, S. crassipalpis,* and three strains of *S. bullata.* Environmental cues of daylength and temperature, water content of the larval medium, and the sex of the animal determine the induction of diapause. Termination of diapause is temperature dependent.

2. Daylength is of primary importance for induction. Diapause is completely averted when adult mothers and larvae are maintained under a long daily photophase or continuous light at 25° C. Short-day exposure of the adults and larvae at 25° induces a high incidence of diapause. However, if short-day is received by only the larvae, diapause is absent, and adult short-day without subsequent larval short-day produces a low diapause incidence. The maximum diapause response is observed when adults are maintained under a short daily photophase at 25°, and larvae are reared at a short daily photophase at 17°.

3. Critical daylength for wild populations of *S. bullata* from Illinois and Missouri is 13½ hours at 25°.

4. A decrease in larval temperature from 28° to 17° C increases the incidence of pupal diapause in animals reared at short-day.

5. Addition of 10% water to larval medium increases diapause incidence 10%.

6. Males enter diapause at a higher rate than females. Sex ratio approaches 1 : 1 in pupae representing 0 and 100% diapause, but lots showing a partial diapause response have a higher percentage of males than females in diapause.

7. Larvae reared under diapause-inducing conditions pupariate over a period of days 3–4 times greater than larvae reared under non-diapause conditions; mean day of pupariation is 4–5 days later in diapause batches. The incidence of pupal diapause increases with an increase in the delay of pupariation.

8. Photoperiod is ineffective in terminating diapause.

9. At constant temperatures the duration of diapause decreases with an increase in temperature. A combination of 17° and 25° provides a shorter diapause than constant exposure to either temperature.

10. Significant differences are found in the diapause responses observed with

different species of *Sarcophaga;* wild strains of *S. bullata* also differ from each other and differ greatly from a lab strain of the same species.

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